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The Impact of a 24-h Ultra-Marathon on Circulatory Endotoxin and Cytokine Profile

Authors

S. K. Gill¹, J. Hankey¹, A. Wright¹, S. Marczak¹, K. Hemming¹, D. M. Allerton², P. Ansley-Robson², R. J. S. Costa³

Affiliations

¹ Sport & Exercise Science Applied Research Group, Coventry University, Coventry, United Kingdom

² Faculty of Health & Life Sciences, Northumbria University, Newcastle upon Tyne, United Kingdom

³ Nutrition and Dietetics, Monash University, Notting Hill, Australia

Key words

- inflammatory
- cytokinaemia
- endotoxaemia
- sleep deprivation
- energy balance
- physical exertion

Abstract



The study aimed to determine circulatory endotoxin concentration, cytokine profile, and gastrointestinal symptoms of ultra-endurance runners (UER, n=17) in response to a 24-h continuous ultra-marathon competition (total distance range: 122–208 km) conducted in temperate ambient conditions (0–20°C) in mountainous terrain. Body mass and body temperature were measured, and venous blood samples were taken before and immediately after competition. Samples were analysed for gram-negative bacterial endotoxin, C-reactive protein, cytokine profile, and plasma osmolality. Gastrointestinal symptoms were also monitored throughout competition. Mean exercise-induced body mass loss was (mean±SD) 1.7±1.8% in UER. Pre- and post-

competition plasma osmolality in UER was 286±11 mOsmol·kg⁻¹ and 286±9 mOsmol·kg⁻¹, respectively. Pre- to post-competition increases (p<0.05) were observed for endotoxin (37%), C-reactive protein (2832%), IL-6 (3436%), IL-1β (332%), TNF-α (35%), IL-10 (511%), and IL-8 (239%) concentrations in UER, with no change in the control group (CON; n=12) observed (p>0.05). Gastrointestinal symptoms were reported by 75% of UER, with no symptoms reported by CON. IL-10 (r=0.535) and IL-8 (r=0.503) were positively correlated with gastrointestinal symptoms. A 24-h continuous ultra-marathon competition in temperate ambient conditions resulted in a circulatory endotoxaemia and pro-inflammatory cytokinaemia, counteracted by a compensatory anti-inflammatory response.

Introduction



The human gut is colonised by a plethora of microorganisms, which have the potential to promote either beneficial or harmful effects on gastrointestinal integrity, function, and overall health [18,22,30]. In the attempt to protect the internal body environment, the intestinal epithelial lining plays a crucial role in preventing harmful microorganisms, some of which present pathogenic properties (e.g., gram negative endotoxins, such as lipopolysaccharides), from entering portal and systemic circulation [25,27]. Prolonged physical exertion however has previously been shown to disturb intestinal epithelial integrity, resulting in increased epithelial permeability, endotoxin leakage, and a subsequent cytokine-mediated systemic inflammatory response similar to that of an acute infectious episode [5,6,11,25].

Most recently, research investigating circulatory endotoxin and cytokine profile during a 230 km multi-stage ultra-marathon in hot ambient condi-

tions (32–40°C), observed a modest circulatory endotoxaemia at rest (60 pg·ml⁻¹ average increase) and after each stage completion (30 pg·ml⁻¹ average increase), accompanied by amplified pro-inflammatory cytokine responses throughout competition (rest: IL-6 152%, IL-1β 95%, TNFα 168%, and IFNγ 102%; and pre- to post-stage: IL-6 238%, IL-1β 64%, TNFα 101%, and IFNγ 39%), despite overnight recovery between stages [11]. Interestingly, the pro-inflammatory cytokine responses appeared to be counteracted by a compensatory anti-inflammatory cytokine cascade (rest: IL-10 1271% and IL-1ra 106%; and pre- to post-stage: IL-10 1100% and IL-1ra 207%). These findings support the proposition that performing physical exertion in hot ambient conditions poses a greater threat to gastrointestinal integrity and function, leading to enhanced intestinal endotoxin permeability and episodes of clinical significance with fatal outcomes being reported (i.e., heat stroke and systemic inflammatory response syndrome) in military, occupation and sport populations [1,23,32,36,43,60,62]. This is

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Correspondence

Dr. Ricardo J. S. Costa, PhD

Nutrition and Dietetics
Monash University
Level 1, 264 Ferntree Gully
Road
3168 Notting Hill
Australia
Tel.: +61/3/99024 270
Fax: +61/3/99024 270
ricardo.costa@monash.edu

not surprising taking into account that exertional-heat stress has the potential to exacerbate splanchnic hypoperfusion and ischemia more than exertional stress in thermoneutral environments [58,61], and thus induce greater disturbances to gastrointestinal integrity and cytokine profile. Indeed, previous laboratory controlled studies have consistently observed greater cytokine responses (e.g., IL-6, TNF α , IL-1 α , and IL-10) in hot (32–39°C) conditions, compared with more temperate (15–22°C) conditions [8,39,46,52,54]; but the degree of impact from intestinal originated endotoxins was not confirmed. Such observations indicate that heat stress appears to play a role in the degree of systemic cytokinaemia observed after physical exertion. Moreover, systemic endotoxin and cytokine responses have also been associated with symptomatic manifestations of gastrointestinal distress [24,25,60] commonly associated with prolonged exposure to exertional and exertional-heat stress [37,40,42,45].

Even though exercising in hot ambient temperatures per se appears to disturb intestinal integrity, promote endotoxin leakage, and augment cytokine-mediated inflammatory responses, it is plausible that extreme ultra-endurance competition, in the absence of heat-stress, but with prolonged exposure to a multitude of other stressors (e.g., prolonged physical exertion, sleep disturbances, compromised hydration and (or) nutritional status) [12,13], may also have the potential to impact gastrointestinal integrity, as well as the subsequent cytokine-mediated inflammatory cascade in a similarly negative manner. For example, increased splanchnic jarring associated with physical exertion, especially prolonged periods of running, has been shown to induce a greater intestinal burden and injury due to mechanical trauma [44,45]. Whereas a pronounced cytokine (i.e., IL-6 and somnogenic cytokines IL-1 β , TNF α , and IFN γ) profile is commonly seen after acute periods of sleep deprivation [15,51] and compromised energy (especially carbohydrate energy) status [33,47].

From a practical perspective, single- and multi-stage ultra-endurance events have increased in popularity over the past decade, yet research into the physiological demands and immune responses to such an extreme sport is limited [31]. Therefore, to date, the short and long-term effects of such ultra-endurance exposure on gastrointestinal integrity and immune responses have not yet been established. It is plausible that ultra-endurance runners competing in such extreme events are potentially a high-risk population group for pronounced acute and long-term gastrointestinal and immune dysfunction, leading to the development of clinically and (or) sub-clinically significant episodes, including gastrointestinal symptoms, gastrointestinal diseases, chronic systemic inflammation, autoimmune diseases, chronic fatigue and underperformance syndrome [7,29,47,50]. With this in mind, the aims of the current study were to determine circulatory endotoxin concentration and cytokine profile of ultra-endurance runners in response to a 24-h continuous ultra-marathon competition conducted in temperate ambient conditions; and additionally to determine the relationship between these responses with gastrointestinal symptoms. Taking into account the nature of the event, it was hypothesised that a pronounced circulatory endotoxaemia would be seen after the event, and a pro-inflammatory cytokinaemic response would mirror the endotoxaemia. Additionally, it was hypothesised that correlations between circulatory responses and gastrointestinal symptoms would be seen.

Methods



Setting and participants

The study was conducted during the 2011 and 2012 Glenmore24 Trail Race (www.glenmore24.com), held during the first week of September, in the Cairngorms National Park, Scottish Highlands, UK (ambient temperature range: 0–20°C, relative humidity range: 54–82%). The 24-h continuous ultra-marathon was conducted on a 6 km looped-course on a variety of terrains; including off-road trails, paths, and grassland. Distance covered by participants ranged between 122 km to 208 km at an estimated average intensity of 7.0 ± 1.3 METs (SenseWear 7.0, Bodymedia, PA, Pittsburgh, USA), and in an altitude averaging 342 m above sea level. After ethical approval from the Coventry University Ethics Committee that conforms with the 2008 Helsinki Declaration for Human Research Ethics and ethical standards of the International Journal of Sports Medicine [21], a convenience sampling observational cohort was studied, whereby 25 out of 48 ultra-endurance runners (UER) entering the event volunteered to participate in the study; however, complete blood sampling and biomarker data were only achieved in $n=17$ (male $n=14$, female $n=3$: age 40 ± 7 years, height 177 ± 9 cm, body mass 78.1 ± 11.9 kg). Additionally, 17 individuals who did not compete (absence of exercise stress), but were present at the race location, volunteered to participate in the study as part of the control (CON) group for comparative purposes, however complete blood sampling and biomarker data were only achieved in $n=12$ (male $n=4$, female $n=8$: age 30 ± 12 years, height 169 ± 10 cm, body mass 68 ± 13 kg). All participants reported no illness and (or) infection in the 12-weeks leading up to the ultra-marathon. Additionally, no anti-inflammatory agents of any form were consumed by all participants in the week leading up and during competition.

Study design and data collection

Within the hour prior to commencement of competition (11:00 h to 12:00 h), body mass was determined using calibrated electronic scales (BF510, Omron Healthcare, Ukyo-ku, Kyoto, Japan) placed on a hard levelled surface. Participants were then required to sit in an upright position for 10 min before tympanic temperature (T_{tym} ; Braun Thermoscan, Kronberg, Germany) was determined and whole blood collected for all participants (UER and CON). Whole blood samples were collected by venepuncture without venostasis from an antecubital vein using a 21G butterfly syringe into one lithium heparin (6 ml, 1.5 IU·ml $^{-1}$ heparin; Becton Dickinson, Oxford, UK) and one K $_3$ EDTA (6 ml, 1.6 mg·ml $^{-1}$ of ethylenediaminetetraacetic acid; Becton Dickinson, Oxford, UK) vacutainer tube. Body mass was re-measured in those participants who needed to urinate prior to the start of competition. Immediately after competition (12:00 h the following day) and before any foods or fluids could be consumed, body mass and T_{tym} were measured, followed immediately by blood sampling, identical to pre-competition procedures. Additionally, within the hour after competition, trained dietetic researchers conducted a standardised structured interview on participants to ascertain total foods and fluids ingested during the ultra-marathon, as previously reported [12,13]. Severe gastrointestinal symptoms [42] were also explored at this time through a research-generated symptomology tool by trained researchers.

Dietary analysis and hydration status

Total energy and water intake through foods and fluids were determined and analysed through Dietplan 6 dietary analysis software (v6.60, Forestfield Software, Horsham, West Sussex, UK). A comprehensive description of the dietary assessment and analysis technique used can be viewed in Costa et al. [10]. Energy expenditure was measured by a triaxial accelerometer, which also included measurements of heat flux, skin temperature and galvanic skin responses (SenseWear 7.0, Bodymedia, PA, Pittsburgh, USA), as has been used previously to aid in nutritional intervention during a mountain-based multi-stage ultra-marathon [4]. Pre- and post-competition plasma osmolality (P_{Osmol}) was determined from 50 μl lithium heparin plasma samples in duplicate by freeze-point osmometry (Osmomat 030, Gonotec, Germany), as recommended previously [53]. The coefficient of variation (CV) for P_{Osmol} was 3.5%.

Blood sample collection and analysis

Blood samples were immediately centrifuged and plasma aliquoted into eppendorfs and stored frozen initially at -20°C during the ultra-marathon competition, prior to transferring to -80°C storage after completion of the experimental procedure. Whole blood (lithium heparin) haemoglobin concentration and haematocrit were used to estimate changes in plasma volume relative to pre-competition, as previously reported [9]. All blood parameters were corrected for changes in plasma volume. Circulatory concentrations of C-reactive protein (CRP) (eBioscience, Hatfield, UK), IL-6, TNF- α , IL-1 β , IFN- γ , IL-10, and IL-8 (human pro-inflammatory 7-plex ultra-sensitive kit (K15008C-1), MesoDiscovery, Gaithersburg, MD, USA) were determined by ELISA using K_3EDTA plasma as per manufacturer's instructions. Gram-negative bacterial endotoxin concentration was determined by limulus amoebocyte lysate (LAL) chromogenic endpoint assay using K_3EDTA plasma (HIT302, Hycult Biotech, Uden, Netherlands) as per manufacturer's instructions. In short, 20 μl of sample was diluted in 380 μl of endotoxin-free water, and then incubated at 75°C for 10 min. Once at room temperature, 50 μl of standards, blank, positive control, and samples were added to plate wells in duplicate. To enhance assay validity, background plate reading without LAL reagent was performed at OD 405 nm. 50 μl LAL reagent was then added. Plate was covered and incubated at 22°C for 30 min, followed by reading at OD 405 nm. Concentration was calculated by plotting the absorbance against standards in a linear regression curve and eliminating background error. The assay was performed using endotoxin-free and dypyrrogenated consumables in a sterile laboratory. All plasma variables analysed were individually run on the same day, with standards and controls on each plate, and each participant assayed on the same plate. The intra-assay coefficient of variation for plasma variables analysed was $\leq 14.7\%$.

Data analysis

Data in text are presented as mean \pm standard deviation (SD), otherwise specified. For clarity, data in figures are presented as mean \pm standard error of the mean (SEM). Due to the commonly large individual variation in cytokine responses [55], data in tables are presented as mean and 95% confidence interval (CI) for mean (lower and upper boundary). Data were processed and analysed in SPSS for Windows (SPSS v.17.0.2, Illinois, US). Prior to data analysis, outlying values for all variables were detected through box-plot analysis and appropriately removed. Diagnos-

tic checks (Shapiro-Wilks test of normality and Levene's homogeneity of variance test) were performed prior to analysis. Where data violated the assumption of normality, data were log transformed, and the transformed data were analysed using parametric statistics, verified against non-parametric equivalents where appropriate. Paired-sample t-tests were applied to determine pre- to post-competition variable differences; while independent-sample t-tests were applied for group comparisons (UER vs. CON, and distance covered ($< 160\text{ km}$ vs. $\geq 160\text{ km}$)) [38,41]. Due to insufficient female ultra-endurance runners sample size, statistical analysis was not viable on this occasion, Spearman's rank correlation analysis was used to assess associations between blood-borne variables with perceived gastrointestinal symptoms. Individual participant ratios between IL-1 β and TNF- α with IL-10 were calculated to determine the pro- to anti-inflammatory cytokine balance. Descriptive statistics were used to explore severe gastrointestinal symptoms. The acceptance level of significance was set at $p < 0.05$.

Results



Energy balance and hydration status

Total energy intake and energy expenditure over the 24-h period was 21 ± 12 and 53 ± 11 MJ for UER, and 12.4 ± 1.3 and 13.6 ± 4.9 MJ for CON, respectively. Significant body mass loss occurred pre- to post-competition ($p = 0.001$) in UER (pre-competition: $78.1 \pm 11.9\text{ kg}$, post-competition: $76.8 \pm 12.0\text{ kg}$, $1.7 \pm 1.8\%$). No significant difference in P_{Osmol} was observed pre- to post-competition in UER (pre-competition: $286 \pm 11\text{ mOsmol} \cdot \text{kg}^{-1}$, post-competition: $286 \pm 9\text{ mOsmol} \cdot \text{kg}^{-1}$) and remained within normal clinical reference range [59]. Compared with CON, P_{Osmol} pre- and post-competition was lower in UER ($p = 0.05$ and $p < 0.001$, respectively).

Tympanic temperature

Tympanic temperature (T_{tymp}) was within normal range pre- and post-stage (36.0 – 37.5°C) in UER and CON, with no difference between pre- and post-stage T_{tymp} observed in both groups. No difference in T_{tymp} was observed between UER and CON.

Circulatory gram-negative bacterial endotoxin concentration

A pre- to post-competition increase in circulatory endotoxin concentration (overall mean change: 37%) was observed in UER ($p = 0.009$; ● Fig. 1), with no significant change in CON evident ($p = 0.005$ vs. UER). No difference in circulatory endotoxin concentration was observed for distance covered.

Plasma C-reactive protein concentration

A pre- to post-competition increase in plasma CRP concentration (2832%) was observed in UER ($p < 0.001$; ● Fig. 2), with no significant change in CON evident ($p < 0.001$ vs. UER). No difference in plasma CRP concentration was observed for distance covered.

Plasma interleukin-6 concentration

A pre- to post-competition increase in plasma IL-6 concentration (3436%) was observed in UER ($p < 0.001$; ● Table 1), with no significant change in CON evident ($p < 0.001$ vs. UER). Post-competition plasma IL-6 concentration was higher ($p = 0.018$) in UER

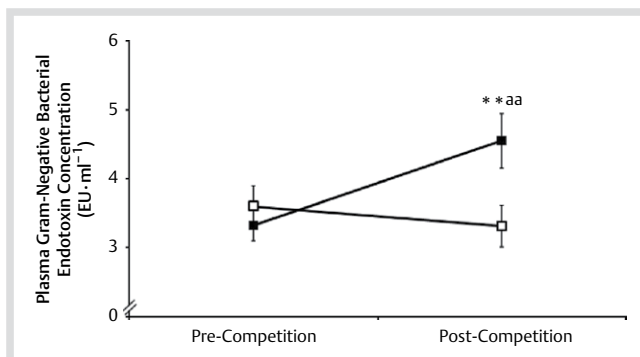


Fig. 1 Circulatory gram-negative bacterial endotoxin concentration of ultra-endurance runners (UER) participating in a 24-h continuous ultra-marathon. Mean \pm SEM: UER (closed squares; $n=17$) and CON (open squares; $n=12$). * $p<0.01$ vs. pre-competition; aa $p<0.01$ vs. CON.

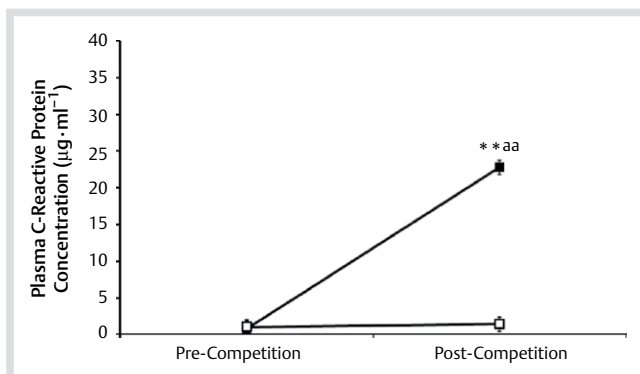


Fig. 2 Plasma C-reactive protein concentration of ultra-endurance runners (UER) participating in a 24-h continuous ultra-marathon. Mean \pm SEM: UER (closed squares; $n=17$) and CON (open squares; $n=12$). * $p<0.01$ vs. pre-competition; aa $p<0.01$ vs. CON.

totalling a distance ≥ 160 km compared to UER totalling a distance < 160 km (Table 2).

Plasma interleukin-1 beta concentration

A pre- to post-competition increase in plasma IL-1 β concentration (332%) was observed in UER ($p=0.05$; Table 1), with no significant change in CON evident ($p=0.032$ vs. UER). No difference in plasma IL-1 β concentration was observed for distance covered.

Plasma tumor necrosis factor alpha concentration

A pre- to post-competition increase in plasma TNF- α concentration (35%) was observed in UER ($p<0.001$; Table 1), with no significant change in CON evident. No difference in plasma TNF- α concentration was observed for distance covered.

Plasma interferon gamma concentration

No significant change in pre- to post-competition plasma IFN- γ concentration was observed in UER or CON. No difference in plasma IFN- γ concentration was observed for distance covered.

Plasma interleukin-10 concentration

A pre- to post-competition increase in plasma IL-10 concentration (511%) was observed in UER ($p<0.001$; Table 1), with no significant change in CON evident ($p<0.001$ vs. UER). Post-competition plasma IL-10 concentration was higher ($p=0.002$) in

Table 1 Plasma cytokine profile of ultra-endurance runners (UER) before and immediately after a 24-h continuous ultra-marathon competition.

	Pre-Competition	Post-Competition
Plasma IL-6 concentration (pg·ml ⁻¹)		
UER	0.4 (0.3 to 0.5)	14.5 (9.3 to 19.7) * * aa
CON	0.6 (0.2 to 0.9)	1.8 (0.5 to 3.1)
Plasma IL-1 β concentration (pg·ml ⁻¹)		
UER	0.1 (0.0 to 0.3)	0.6 (0.1 to 1.1) * a
CON	0.0 (0.0 to 0.1)	0.0 (0.0 to 0.1)
Plasma TNF- α concentration (pg·ml ⁻¹)		
UER	2.8 (2.5 to 3.2)	3.8 (3.5 to 4.2) * *
CON	2.6 (1.8 to 3.3)	3.2 (2.4 to 4.0)
Plasma IFN- γ concentration (pg·ml ⁻¹)		
UER	1.0 (0.6 to 1.4)	1.2 (0.3 to 2.2)
CON	1.1 (0.6 to 1.6)	1.1 (0.6 to 1.6)
Plasma IL-10 concentration (pg·ml ⁻¹)		
UER	2.1 (1.3 to 2.9)	12.8 (7.3 to 18.2) * * aa
CON	1.6 (0.1 to 3.2)	1.7 (0.1 to 3.3)
Plasma IL-8 concentration (pg·ml ⁻¹)		
UER	11.4 (9.4 to 13.4)	38.7 (26.3 to 51.1) * * aa
CON	14.0 (10.2 to 17.7)	14.2 (11.1 to 17.2)

Mean and 95% CI (lower and upper bound): UER ($n=17$) and CON ($n=12$). * $p<0.05$ and * * $p<0.01$ vs. pre-competition, ^a $p<0.05$ and ^{aa} $p<0.01$ vs. CON

UER totalling a distance ≥ 160 km compared to UER totalling a distance < 160 km (Table 2).

Plasma interleukin-8 concentration

A pre- to post-competition increase in plasma IL-8 concentration (239%) was observed in UER ($p<0.001$; Table 1), with no significant change in CON evident ($p<0.001$ vs. UER). A trend ($p=0.102$) for higher post-competition plasma IL-8 concentration was observed in UER totalling a distance ≥ 160 km compared to UER totalling a distance < 160 km (Table 2).

Pro-inflammatory to anti-inflammatory cytokine ratio

A pre- to post-competition decrease in TNF- α :IL-10 ratio (72%; $p<0.001$) was observed in UER, with no significant change in CON evident ($p=0.011$ vs. UER). While a 32% decrease in IL-1 β :IL-10 ratio was also observed in UER ($p=0.063$ vs. CON), but this failed to reach significance ($p>0.05$). Post-competition TNF- α :IL-10 ratios were substantially lower ($p=0.008$) in UER totalling a distance ≥ 160 km compared to UER totalling a distance < 160 km.

Gastrointestinal symptoms

Severe gastrointestinal symptoms were a common feature amongst the cohort sampled for endotoxin and cytokine responses; with 75% of the cohort reporting at least one severe gastrointestinal symptom (including 63% reporting nausea) during competition. Greater reports of severe gastrointestinal symptoms were reported by UER totalling a distance ≥ 160 km compared to UER totalling a distance < 160 km ($p=0.012$). Spearman's rank correlation analysis showed a positive correlation between reported rates of gastrointestinal symptoms and plasma IL-10 ($r=0.535$, $p=0.034$) and IL-8 ($r=0.503$, $p=0.047$) concentrations.

Table 2 Pre- and post-competition circulatory gram-negative bacterial endotoxin concentration, plasma C-reactive protein concentration, and plasma cytokine profile of ultra-endurance runners who completed ≥ 160 km and < 160 km during a 24-h continuous ultra-marathon competition.

	Pre-Competition	Post-Competition
Circulatory gram-negative bacterial endotoxin concentration (EU · ml ⁻¹)		
≥ 160 km	3.5 (2.7 to 4.3)	4.5 (3.6 to 5.2)
< 160 km	3.1 (2.5 to 3.7)	4.7 (3.3 to 6.0)
Plasma C-reactive protein concentration ($\mu\text{g} \cdot \text{ml}^{-1}$)		
≥ 160 km	0.5 (0.2 to 0.8)	22.2 (19.4 to 26.1)
< 160 km	1.0 (0.0 to 2.1)	23.4 (21.1 to 25.8)
Plasma IL-6 concentration (pg · ml ⁻¹)		
≥ 160 km	0.5 (0.2 to 0.6)	19.8 (12.0 to 30.4) ^a
< 160 km	0.4 (0.3 to 0.5)	8.5 (5.6 to 11.5)
Plasma IL-1 β concentration (pg · ml ⁻¹)		
≥ 160 km	0.3 (0.0 to 0.7)	0.6 (0.0 to 1.6)
< 160 km	0.0 (0.0 to 0.0)	0.6 (0.0 to 1.4)
Plasma TNF- α concentration (pg · ml ⁻¹)		
≥ 160 km	2.7 (1.8 to 3.4)	3.8 (3.3 to 4.4)
< 160 km	3.0 (2.7 to 3.3)	3.8 (3.2 to 4.5)
Plasma IFN- γ concentration (pg · ml ⁻¹)		
≥ 160 km	1.0 (0.3 to 1.5)	1.2 (0.1 to 1.7)
< 160 km	0.7 (0.2 to 1.1)	0.5 (0.2 to 0.7)
Plasma IL-10 concentration (pg · ml ⁻¹)		
≥ 160 km	2.0 (0.8 to 3.1)	19.7 (10.1 to 27.0) ^{aa}
< 160 km	2.2 (0.7 to 3.7)	4.9 (3.3 to 6.5)
Plasma IL-8 concentration (pg · ml ⁻¹)		
≥ 160 km	11.5 (9.0 to 15.3)	47.7 (24.8 to 76.6) [§]
< 160 km	11.3 (8.0 to 14.6)	28.5 (22.3 to 34.8)

Mean and 95% CI (lower and upper bound): ≥ 160 km (n=9) and < 160 km (n=8).

^ap<0.05 and ^{aa}p<0.01 vs. < 160 km, [§] p=0.102 vs. < 160 km

Discussion

The current study aimed to determine circulatory endotoxin concentration and cytokine profile of runners participating in a 24-h continuous ultra-marathon competition; and additionally to determine the relationship between these responses with gastrointestinal symptoms. Findings confirm that in the absence of heat-stress, but with prolonged exposure to other multi-stressors, resultant endotoxaemia with accompanying cytokinaemia were characteristic of an acute infectious episode. Severe gastrointestinal symptoms were a common feature, with higher symptom occurrence associated with greater compensatory anti-inflammatory (i.e., IL-10) and immune activation (i.e., IL-8) responses. Additionally, post-competition IL-6, IL-10, and IL-8 responses were higher and TNF- α :IL-10 ratio was lower in those ultra-endurance runners totalling a distance ≥ 160 km.

In comparison to a 230 km multi-stage ultra-marathon conducted in hot ambient conditions (32–40°C), whereby resting plasma CRP concentration increased 889% and circulatory endotoxin concentration peaked at 21% by Stage 5 [11], plasma CRP and circulatory endotoxin concentration in the current study were markedly more pronounced despite the absence of heat stress, increasing 2832% and 37% post-competition, respectively. These results suggest that amplified CRP concentrations likely reflect other multi-factorial influences, such as the degree of the exertional stress, sleep deprivation, energy deficit, and bacterial endotoxin presence in circulation simultaneously. Whereas, the endotoxin leakage was likely induced by disturbances to intestinal epithelial integrity (i.e., splanchnic hypoperfusion, ischemia, and mechanical trauma), associated with the

physical exertion volume; despite endotoxin leakage being more commonly studied amongst exertional-heat stress protocols [2,3]. Due to practical limitations in monitoring parameters after competition (i.e., participant follow-up at race location during the acute and prolonged recovery period), the current study was not able to determine the recovery time course of CRP. However, such responses have been shown to remain elevated above pre-exercise values for a considerable period of time (i.e., up to 19 days after an Ironman triathlon event) [34].

Despite temperate ambient conditions, the current study observed increases in pro-inflammatory cytokine responses post-competition, similar to that of an acute infectious episode; whereby IL-6, IL-1 β , and TNF- α increased 3436%, 332%, and 35%, respectively. These results are similar to previous field-based studies that have observed cytokinaemia during exertional-heat stress [2,3,24,56]. For example, increases in IL-6 (152%), IL-1 β (95%), TNF- α (168%), and IFN γ (102%) were consistently observed post-stage during a 230 km multi-stage ultra-marathon conducted in hot ambient conditions [11]. Acute pro-inflammatory response is commonly accompanied by compensatory anti-inflammatory (i.e., 511% increase in IL-10) and immune activation (i.e., 239% increase in IL-8) responses. As such, the observed decreases in TNF- α :IL-10 and IL-1 β :IL-10 ratios in the current study suggest that the anti-inflammatory properties of IL-10 may have restricted the magnitude of pro-inflammatory cytokine responses induced by the extreme nature of this ultra-marathon. Notably, those ultra-endurance runners totalling a distance of ≥ 160 km had significantly greater post-competition IL-6 and IL-10 responses (overall mean: 19.8 pg · ml⁻¹ and 19.7 pg · ml⁻¹, respectively) compared to those ultra-endurance runners totalling a distance < 160 km (overall mean: 8.5 pg · ml⁻¹ and 4.9 pg · ml⁻¹, respectively). Additionally, post-competition TNF- α :IL-10 ratio was substantially lower in ultra-endurance runners totalling a distance ≥ 160 km compared to ultra-endurance runners totalling a distance < 160 km.

Results from the current study suggest that in well-trained individuals, where the exertional stress is well tolerated, compensatory anti-inflammatory responses may offset potential clinically significant episodes associated with profound and prolonged cytokine profile disturbance [17,19,35,57], thus acting as a safeguard. Indeed, disturbances to cytokine profile have been linked to the aetiology of gastrointestinal and autoimmune diseases [7,16], while the fatigue inducing properties of certain cytokines, especially IL-6, have recently been confirmed and established [29,48–50]. Previous research has shown that illness-prone individuals have an altered cytokine response (i.e., pro- to anti-inflammatory balance) after a standardised bout of treadmill running compared with healthy runners who have a more proportionally regulated cytokine response [14]. Therefore, it is plausible that in illness-prone individuals partaking in prolonged physical exertion who experience impaired cytokine regulation (i.e., over-exaggerated cytokine-mediated inflammatory response) not adequately counteracted by anti-inflammatory responses, the resulting cytokine imbalance may potentially contributing to pathophysiology [17]. Investigation into the role of challenging compensatory anti-inflammatory responses through internal (e.g., endotoxin challenge) and external (e.g., physical strain) mechanisms is warranted, and may act to strengthen tolerance to exertional exposure with or without additional stressors (e.g., heat stress, sleep deprivation, and/or compromised nutrition) in illness-prone individuals.

As previously reported, due to practical limitations, the current study was not able to determine the recovery time course of the cytokine profile. Previous ultra-endurance studies (e.g., long-distance triathlon and ultra-marathon running) have however observed variations in cytokine recovery. For example, IL-6 and TNF- α returned to baseline by 24 h after a 50 km ultra-marathon [28]; whilst IL-6 returned to baseline values after 16 h, with no significant change observed in TNF- α after a long-distance triathlon [24]. Furthermore, on cessation of 2 endurance events of similar duration (i.e., long-distance triathlon and 100 km running event), IL-6, IL-10, and IL-1ra peaked after competition, returning to baseline values 7 days after the events [20]. Whereas, after a long distance triathlon, IL-6 remained elevated on day 1 (345%) and day 5 (79%); while IL-10 was elevated on day 1 (37%), declining by 4% below pre-competition concentrations on day 5 [34].

Circulatory endotoxin and pro-inflammatory cytokine responses seen in the current study have previously been associated with symptomatic manifestations of gastrointestinal distress [24–26,60]. In accordance with a recent multi-stage ultra-marathon study [11], despite 58% of UER sampled for endotoxin and cytokine responses reporting severe gastrointestinal symptoms during competition, and reporting being greater in ultra-endurance runners totalling a distance ≥ 160 km compared to ultra-endurance runners totalling a distance < 160 km, no relationships between gastrointestinal symptoms with circulatory endotoxin and pro-inflammatory cytokine concentrations were observed in the current study. Interestingly, an association between the reported number of severe gastrointestinal symptoms and plasma concentrations of anti-inflammatory IL-10 ($r=0.535$) and immune activator IL-8 ($r=0.503$) were observed. These results suggest that alterations to intestinal motility and mechanical trauma (i.e., repetitive jarring) associated with the prolonged running period may have promoted intestinal mucosa and epithelial disturbance and potential damage [44,45], inducing gastrointestinal symptoms and stimulating immune responses into protection and repair; and not necessarily that endotoxaemia and cytokinaemia causes symptoms.

Conclusion

In conclusion, a 24-h continuous ultra-marathon competition conducted in temperate ambient conditions with inclusion of multi-stressors (i.e., sleep deprivation and energy deficit) resulted in endotoxaemia with accompanying cytokinaemia characteristic of an acute infectious episode. Gastrointestinal symptoms were commonly reported amongst ultra-endurance runners, with higher symptom occurrence associated with greater compensatory anti-inflammatory responses and immune activation suggesting that alterations to intestinal motility and mechanical trauma may have promoted intestinal mucosa and epithelial disturbance, inducing gastrointestinal symptoms. Further research is required to clarify the impact of over-exaggerated cytokine-mediated inflammatory responses, similar to those observed in the current study, on the potential long-term health implications in 'high-risk' illness-prone individuals.

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